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Randomized noninferiority clinical trial evaluating 3 commercial dry cow mastitis preparations: I. Quarter-level outcomes

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ABSTRACT

The study objective was to compare the efficacy of 3 commercial dry cow mastitis formulations regarding quarter-level prevalence of intramammary infections (IMI) postcalving, cure of preexisting infections over the dry period, prevention of new infections during the dry period, and risk for a clinical mastitis case between calving and 100 d in milk (DIM). A total of 1,091 cows (4,364 quarters) from 6 commercial dairy herds in 4 different states (California, Iowa, Minnesota, and Wisconsin) were enrolled and randomized to 1 of the 3 treatments at dry-off: Quartermaster (QT; 1,000,000 IU of procaine penicillin G and 1 g of dihydrostreptomycin; Pfizer Animal Health, New York, NY), Spectramast DC (SP; 500 mg of ceftiofur hydrochloride; Pfizer Animal Health), or ToMorrow Dry Cow (TM; 300 mg of cephapirin benzathine; Boehringer Ingelheim Vetmedica Inc., St. Joseph, MO). Quarter milk samples were collected for routine bacteriological culture before dry cow therapy treatment at dry-off, 0 to 6 DIM, and 7 to 13 DIM and an on-farm record-keeping system was used to retrieve data on clinical mastitis cases. Noninferiority analysis was used to evaluate the effect of treatment on the primary outcome, risk for a bacteriological cure during the dry period. Multivariable logistic regression techniques were used to describe the effect of treatment on risk for presence of IMI postcalving and risk of a new IMI during the dry period. Cox proportional hazards regression was used to describe the effect of treatment on the risk and time for quarters to experience an episode of clinical mastitis between calving and 100 DIM. The overall crude quarter-level prevalence of infection at dry-off was 19.2%. The most common pathogen isolated from milk samples at dry-off was coagulase-negative *Staphylococcus*, followed by *Aerococcus* spp. and other *Streptococcus* spp. Noninferiority analysis showed no effect of treatment on risk for a cure between dry-off and calving [least squares means (LSM): QT = 93.3%, SP = 92.6%, and TM = 94.0%] and secondary analysis showed no effect of treatment on risk for presence of an IMI at 0 to 6 DIM (LSM: QT = 16.5%, SP = 14.1%, and TM = 16.0%), risk for development of a new IMI between dry-off and 0 to 6 DIM (LSM: QT = 14.8%, SP = 12.3%, and TM = 14.2%), or risk of experiencing a clinical mastitis event between calving and 100 DIM (LSM: QT = 5.3%, SP = 3.8%, and TM = 4.1%). In conclusion, no difference was observed in efficacy among the 3 products evaluated when assessing the aforementioned quarter-level outcomes.

Key words: dry cow mastitis, dry cow therapy, quarter-level outcome

INTRODUCTION

The dry period corresponds to a crucial period when lactating cows go through physiological changes to prepare the mammary gland for the next lactation. The importance of mastitis during the dry period has been explored by several authors (Oliver and Mitchell, 1983; Eberhart, 1986; Erskine, 2001). Persistence of preexisting IMI through the dry period and development of new IMI (**NIMI**) during the dry period are 2 important factors that increase the risk for manifestation of clinical mastitis in the next lactation. Estimates of dry cow mastitis incidence rates vary among studies, in part due to differences in definitions of IMI, regional differences, and herd differences. North American studies have estimated the proportion of quarters developing an NIMI during the dry period to range between 8 and 25% (Eberhart, 1986; Godden et al., 2003; Cook et al., 2005). The majority of new infections are subclinical during the dry period, but can flare up as clinical mastitis, usually in early lactation (Green et al., 2002). It has been estimated that 55% of environmental infec-

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tions established early in the dry period persist into the next lactation and can possibly cause clinical flare-ups (Todhunter et al., 1995), and that 52% of all clinical coliform mastitis cases occurring in the first 100 d of lactation may originate during the previous dry period (Bradley and Green, 2000). Smith et al. (1985) reported that the risk for NIMI from environmental pathogens can be 10 times higher during the dry period than during the lactation period.

Blanket dry cow therapy (**DCT**), which refers to the intramammary infusion of all quarters of all cows at dry-off with a long-acting antibiotic, is a procedure recommended by the National Mastitis Council (**NMC**) as mastitis control practice, both for the purpose of curing existing subclinical infections and preventing new infections that could be acquired during the early dry period. North American studies estimate the proportion of quarters infected subclinically at dry-off to vary between 13 and 35% (Oliver and Mitchell, 1983; Godden et al., 2003; Pantoja et al., 2009). Advantages of using DCT include avoidance of milk discard during the lactation period, use of larger doses of antibiotic (so that concentrations can stay above MIC for longer periods of time), and reduction in risk for antibiotic residues in saleable milk. It has been estimated that 72.3% of the US dairy operations use blanket DCT, which corresponds to 81.7% of US dairy cows (USDA-NAHMS, 2008).

According to the 2013 "Milk and Dairy Beef Drug Residue Prevention" manual (National Milk Producers Federation, 2013) 7 commercial dry cow mastitis products are currently approved by the Food and Drug Administration for use in US dairy herds. Milk and meat withhold period, dry period length, claimed spectrum of action, and cost for these products vary considerably. Three commonly used dry cow products in the United States include Quartermaster (**QT**; Pfizer Animal Health, New York, NY), Spectramast DC (**SP**; Pfizer Animal Health), and ToMorrow Dry Cow (**TM**; Boehringer Ingelheim Vetmedica Inc., St. Joseph, MO). Quartermaster is composed of 1,000,000 IU of procaine penicillin G and 1 g of dihydrostreptomycin. It is labeled to reduce the frequency of existing infections and prevent new infections caused by *Staphylococcus aureus*. Milk and meat withholding times are 96 h postcalving, and 60 d postinfusion, respectively. The dry period length is required to be at least 42 d. Spectramast DC is composed of 500 mg of ceftiofur hydrochloride and labeled for subclinical mastitis associated with *Staph. aureus*, *Streptococcus dysgalactiae*, and *Streptococcus uberis*. It has no milk withholding time and the meat withdrawal period is 16 d postinfusion for this product. The required dry period length is 30 d. Finally, TM contains 300 mg of cephapirin and is labeled to be effective on the treatment of mastitis caused by *Streptococcus agalactiae* and *Staph. aureus*, including penicillin-resistant strains. Milk and meat withholding times are 3 d after calving and 42 d after treatment, respectively, and the required dry period length is 30 d.

The efficacy of commercial DCT products compared with negative controls (untreated quarters) has been previously demonstrated for each of these products to receive Food and Drug Administration approval. However, studies comparing efficacy among available DCT products in field conditions in North American dairy herds have been previously lacking. Hallberg et al. (2006) conducted a study to evaluate the efficacy of ceftiofur hydrochloride for the treatment of existing IMI at dry-off and prevention of NIMI during the dry period using a negative control and a positive control (cephapirin benzathine), but that study was not designed to compare efficacy between the 2 antimicrobial formulations used, nor did the authors complete and report statistical analysis comparing the 2 antimicrobial treatments. Furthermore, that study only enrolled cows with an elevated SCC (>400,000 cells/ mL), and so results may not be generalizable to commercial dairy herds wherein blanket DCT is usually applied to all cows. A recent study conducted in Florida compared treatment with SP with treatment with QT at the cow level, but the authors did not report quarter-level outcomes such as risk for new IMI or risk for cure of preexisting IMI (Pinedo et al., 2012). The comparative efficacy of available DCT formulations deserves to be investigated so that producers can make informed science-based decisions when selecting DCT products for use in their herds. The ultimate goal of the current noninferiority multi-herd, multi-state study is to promote judicious drug use while providing producers with needed information to guide the selection of efficacious DCT products, thus promoting cow health and welfare, as well as economic sustainability of the dairy farm.

The study objective was to compare the efficacy of 3 commercial DCT products as measured by quarter-level risk for presence of an IMI postcalving, risk for cure of an IMI during the dry period, risk for development of NIMI over the dry period, and risk for experiencing a clinical mastitis event between calving and 100 DIM. The hypothesis tested was that quarters infused with cephapirin benzathine at the time of dry-off would have a noninferior proportion of quarters cured from preexisting IMI, and would have similar prevalence of IMI postcalving, incidence of NIMI over the dry period, and incidence of clinical mastitis from calving to 100 DIM compared with quarters infused with either ceftiofur hydrochloride or penicillin/dihydrostreptomycin.

MATERIALS AND METHODS

Herd Selection

A noninferiority randomized clinical trial was conducted under Institutional Animal Care and Use Committee (IACUC) approval from each participating University between February 2011 and November 2011 in 6 commercial dairy herds located in California (n = 2), Iowa (n = 1), Minnesota (n = 1), and Wisconsin (n = 2). Herds were located within a reasonable driving distance from the collaborating institution. Inclusion criteria for study herds included to be on a regular DHIA testing program and comply with the study protocol. This convenience sample of herds averaged 2,230 lactating cows (range of 1,050 to 3,600), with an average bulk tank SCC of 242,170 cells/ mL (range of 148,000 to 330,000 cells/ mL), and a rolling herd average of 12,360 kg (range of 10,610 to 13,550 kg; Table 1). Bulk samples were negative on culture for *Mycoplasma* spp. for all 6 herds before initiating the study. All herds routinely used an internal teat sealant at dry off (Orbeseal; Pfizer Animal Health), commercial coliform mastitis vaccines, and blanket DCT.

Cow Enrollment

To be eligible for enrollment, cows had to be in good general health, have 4 functioning quarters, have not received parenteral or intramammary treatment with an antibacterial or antiinflammatory medication during a 30-d period immediately before dry off and show no clinical signs of clinical mastitis on the day of dry-off. All study enrollment and sampling activities were conducted by the authors or University technicians who visited the herd weekly. Cows due to be dried off were brought into the parlor for their last milking and routine DCT. Cow identification numbers were previously assessed and animals were checked for previous medication. Animals were identified while entering the parlor and visually inspected for clinical signs of illness such as very low BCS (<2.0) or moderate to severe lameness. The udder and milk were inspected for signs of clinical mastitis. Eligible cows were randomly allocated to treat all 4 quarters with 1 of the 3 treatments (QT, SP, or TM) according to a previously prepared randomized spreadsheet created in Excel software (2010; Microsoft Corp., Santa Rosa, CA). Randomization was blocked within farms on each day of enrollment.

Routine parlor udder preparation was performed by the farm personnel while study investigators recorded teat end and udder hygiene scores. Although the type of predip used and sequence of predipping and forestripping was not identical among all herds, the general process involved predipping, forestripping, and redipping, leaving the dip on for at least 45 s of contact time, and then wiping teat barrel and teat end dry with a clean cloth towel. Teat end scores ranged from 1 (no teat end crack or callosity) to 4 (cracked teat end; Falkenberg et al., 2003) and udder hygiene scores ranged from 1 (clean) to 4 (dirty; Schreiner and Ruegg, 2003). Following routine udder preparation, sample collectors cleaned and disinfected the teat ends using gauze squares soaked in 70% isopropyl alcohol. Three strippings of fore milk were discarded and duplicate quarter samples were aseptically collected into sterile milk vials previously identified with herd, cow number, quarter, and date. After sample collection (sample 1, **S1**), the routine final milking procedure took place and milk sample vials were placed into a chilled cooler on ice. Immediately following the final milking, all 4 quarters were again scrubbed with alcohol-soaked gauze, the assigned treatment was infused into each of the 4 quarters, and finally the internal teat sealant was infused. All cows were postdipped and moved to their respective dry cow facilities, where usual farm dry cow husbandry and management practices were undertaken.

Postcalving Sampling and Follow-Up

Investigators visited the herds once per week and postcalving duplicate quarter milk samples were collected at 2 different time periods: 0 to 6 DIM (sample

Table 1. Herd descriptors

Item	Herd A	Herd B	Herd C	Herd D	Herd E	Herd F
State	Wisconsin	Wisconsin	Minnesota	Iowa	California	California
RHA[1] (kg)	13,170	12,690	13,550	12,310	10,610	11,790
SCC[2] (cells/mL)	284,000	275,000	236,000	148,000	330,000	180,000
Size (no. of lactating cows)	1,550	1,050	1,650	3,030	2,500	3,600
Housing during dry period	Freestall, pasture	Freestall	Freestall	Open lot	Open lot	Freestall, open lot
Bedding during dry period	Sand	Biosolids	Sand	Corn stalk	Biosolids	Biosolids
Housing during lactation	Freestall	Freestall	Freestall	Freestall	Open lot	Freestall
Bedding during lactation	Sand	Biosolids	Sand	Sand	Biosolids	Biosolids

[1]Milk production annual rolling herd average (RHA).

[2]Bulk tank milk average SCC.

2, **S2**) and 7 to 13 DIM (sample 3, **S3**). The procedure for sample collection was the same as previously described for S1 collected at dry-off. All clinical mastitis events occurring in the first 100 DIM were recorded by farm staff using an on-farm electronic record-keeping system (DairyComp305; Valley Agricultural Software, Tulare, CA) and farm personnel were asked to collect and freeze an aseptic milk sample from the affected quarter at time of detection of a clinical case. Clinical mastitis was defined as visibly abnormal milk accompanied or not by changes in the quarter. Samples were kept frozen (−20°C) at the farm until the next investigator's visit. DairyComp305 software was used to capture electronic DHIA records for all study cows throughout the 100-DIM observation period to provide test-day measures of previous linear score and previous milk production, clinical mastitis events, and death and culling events. The effect of treatment on long-term cow-level outcomes will be reported upon in a companion manuscript.

Laboratory Methods

Milk samples collected on farms were placed on ice and transported back to the local participating laboratory (Laboratory for Udder Health, St Paul, MN; Veterinary Diagnostic Laboratory, Ames, IA; or Dairy Food Safety Laboratory, Tulare, CA), where they were immediately frozen at −20°C until they could undergo bacterial culture. Bacteriological milk culture procedures were standardized as much as possible among the 3 participating laboratories and followed published procedures recognized by the NMC for bovine mastitis (NMC, 1999). Only one of each pair of duplicate quarter samples was routinely selected for microbial culture. The second paired sample was kept frozen in reserve and only cultured in cases where the first sample was contaminated.

Samples to be cultured were thawed to room temperature and 0.01 mL of milk was plated into MacConkey agar and either Factor agar (for those samples submitted to the Minnesota laboratory) or blood agar (for samples submitted to California or Iowa laboratories) using calibrated loops (note: Factor agar is routinely used at the Laboratory for Udder Health from the University of Minnesota, St. Paul, for selective growth of gram-positive organisms; University of Minnesota Laboratory for Udder Health, 2013). Inoculated plates were incubated at 37°C for 48 h and then observed for bacterial growth. For plates with bacterial growth, the number of colonies was recorded for each species isolated, and colonies were reisolated on blood agar for further characterization. Colony morphology, hemolysis pattern, and Gram staining results were described.

Further characterization of gram-positive organisms involved the catalase test reaction to differentiate *Staphylococcus* and *Streptococcus* species, and then coagulase testing. Organisms that were catalase positive and coagulase negative were reported as CNS, whereas catalase- and coagulase-positive organisms were reported as *Staph. aureus*. Catalase-negative organisms had their identity confirmed by the API *Streptococcus* identification system (bioMérieux Vitek Inc., Hazelwood, MO). Pathogens reported as other *Streptococcus* spp. corresponded to subspecies of *Streptococcus* that are less commonly reported in the literature or to pathogens that could not be identified by the API *Streptococcus* system. Gram-positive organisms that were in very low prevalence and pathogens that grew in Factor but not in MacConkey agar and could not be identified were reported as "other gram positives."

Further characterization of gram-negative organisms involved motility testing and then testing with the API20E system (bioMérieux Vitek Inc.). Organisms that could not be identified by the API system were reported as "other gram negatives." Finally, nonbacterial pathogens such as yeast were reported as "others."

If 3 or more pathogens were present in a single sample, it was considered contamination and the duplicate sample was cultured. If the duplicate sample also yielded 3 or more bacterial pathogens, the quarter sample was reported as contaminated. Blinding of the sample collectors or producer at the time of treatment was not possible. However, laboratory personnel were blinded to treatment.

Definitions

Presence of an IMI. An IMI was defined as 1 or more colonies isolated from a 0.01-mL milk sample for all pathogens except for CNS and *Bacillus* spp. For CNS, 2 or more colonies isolated from a 0.01-mL milk sample were needed to establish presence of an IMI (Dohoo et al., 2011). For *Bacillus* spp., an IMI was defined as 5 or more colonies isolated from a 0.01-mL milk sample. Because no peer-reviewed studies exist determining a cutoff point for the latter organism, the definition for IMI for *Bacillus* spp. was established during an informal discussion among mastitis experts (chaired by B. Owens, Louisiana State University Agricultural Center, Hill Farm Research Station, Homer) conducted during the 2011 Mastitis Research Workers' Conference (Nov. 1, 2011, Chicago, IL). A single IMI was defined as the presence of only 1 type of pathogen in the sample, whereas mixed infections corresponded to the presence of 2 different bacterial species.

Bacteriological Cure. A cure was defined as the failure to culture 1 or 2 of the pathogens originally

present at the dry-off sample (Godden et al., 2003) in both postcalving samples (S2 and S3). Quarters with contaminated or missing samples were not included in the analysis.

New IMI. An NIMI was defined as quarters from which no pathogens were recovered at dry-off (S1) but growth was later detected in the first postcalving sample (S2) or a different (new) pathogen was recovered at S2 compared with S1. Quarters that had 1 or both samples contaminated or missing were not included in the analysis. It was possible for the same quarter to experience both a cure and an NIMI.

Statistical Analysis

For the a priori sample size calculation, the primary outcome considered was risk for a cure. The minimum difference in cure rate to declare noninferiority of TM compared with SP or QT was prestated at 10%. To demonstrate noninferiority, a total of 339 cows (1,356 quarters) per group were estimated to be required (assuming $\alpha = 0.025$ and $\beta = 0.2$), along with 10% losses to follow-up and 30% of the quarters infected at dry-off and, therefore, at risk for a cure (noninferiority tests for 2 proportions; Pass 2008; NCSS LLC, Kaysville, UT).

All statistical analyses were conducted using the intent-to-treat approach at the quarter level in SAS (version 9.2: SAS Institute Inc., Cary, NC). Initially descriptive statistics and plots were generated for exploratory data analysis. Basic diagnostic techniques were used to evaluate normality and presence of outliers. Characteristics of cows and quarters assigned to the 3 treatment groups were initially compared at baseline using the chi-squared test and ANOVA.

The effect of treatment on binary outcomes such as risk for presence of IMI, cure, and NIMI were estimated using multivariable logistic regression (generalized linear mixed model) using PROC GLIMMIX, with region included as a fixed effect and herd and cow included as random effects in the model to account for the clustering effects of herds within regions (3 different participating laboratories), cows within herds, and quarters within cows.

Covariates offered to the model included DCT treatment group (forced), cow parity, previous lactation linear score (**LS**), previous lactation total milk production (kg), dry period length (d), teat end score at dry-off and postcalving, and udder hygiene score at dry-off and postcalving. The variables previous lactation LS, previous lactation total milk production, and dry period length were offered as continuous variables. Udder hygiene score was offered as a categorical variable in 4 levels and cow parity was dichotomized in 2 categories: second parity and third-or-greater parities. Teat end score was initially captured in 4 categories but 2 categories were considered in the model: categories 1 and 2 and categories 3 and 4, for the reason that relatively few teat ends scored 4. Univariate analysis was initially conducted between each of the aforementioned variables and the dependent variable of interest, and variables significant at $P < 0.2$ were then carried forward to offer to the full model. Nonsignificant variables were then removed one at a time using a backward stepwise approach, with final significance declared at $P < 0.05$. First-order interactions between DCT treatment group and other remaining main effects were tested and included in the model if significant. Models were compared during the model-building process using the −2 log-likelihood statistics and the final model fit was assessed using PROC LOGISTIC with the Hosmer-Lemeshow goodness-of-fit test.

Noninferiority analysis of the effect of treatment on risk for a bacteriological cure was completed by constructing a figure containing the confidence intervals for the treatment relative to both null (reference treatments, QT, and SP) and the margins of equivalence (Piaggio et al., 2006).

Cox proportional hazards regression (PROC PHREG) was used to describe the effect of DCT treatment on the survival distribution function of quarters experiencing a case of clinical mastitis between calving and 100 DIM (note: no clinical mastitis events were reported by the farm personnel during the dry period). Quarters were considered to be at risk between calving and 100 DIM, with the failure date defined as the date when the quarter was first reported to be affected by a clinical mastitis event. Quarters not reported to experience a clinical mastitis event were classified as censored either at the cow's culling or death date (if before 100 DIM) or at 100 DIM. Clustering at the herd level was controlled for with a COVSANDWICH statement. Covariates offered to the model included DCT treatment group (forced), region (forced), cow parity, previous lactation LS, previous lactation total milk production (kg), dry period length (days), teat end score at dry-off, and udder hygiene score at dry-off. Models were compared during the model-building process using the −2 log-likelihood statistics and the final model fit was assessed plotting the deviance residuals.

RESULTS

A total of 4,364 quarters (1,091 cows) were enrolled in the study between February and April of 2011. Of those, 1,492, 1,396, and 1,476 quarters were allocated to treatment groups QT, SP, and TM, respectively. The treatment groups did not differ at enrollment regarding the following cow-level parameters (overall mean ±

Table 2. Crude prevalence (no., with percentages in parentheses) of intramammary infections for quarters at dry-off and 0 to 6 d in milk by treatment group and overall[1]

	IMI present at dry-off				IMI present at 0 to 6 DIM			
Item	QT	SP	TM	Total	QT	SP	TM	Total
No growth	1,180 (79.4)	1,105 (79.4)	1,140 (77.4)	3,425 (78.7)	1,179 (82.8)	1,100 (84.6)	1,170 (83.1)	3,449 (83.5)
Total IMI	279 (18.8)	253 (18.2)	305 (20.7)	835 (19.2)	219 (15.4)	174 (13.4)	216 (15.3)	609 (14.7)
Single IMI	256 (17.2)	226 (16.5)	275 (18.7)	753 (17.3)	189 (13.3)	146 (11.2)	194 (13.8)	529 (12.8)
Mixed IMI	23 (1.6)	27 (1.9)	30 (2.0)	82 (1.9)	30 (2.1)	28 (2.1)	22 (1.6)	80 (1.9)
Contaminated	28 (1.9)	33 (2.4)	30 (2.0)	91 (2.1)	26 (1.8)	27 (2.1)	22 (1.6)	75 (1.8)
Total quarters	1,487	1,391	1,473	4,351	1,424	1,301	1,408	4,133

[1]QT = Quartermaster (1,000,000 IU of procaine penicillin G and 1 g of dihydrostreptomycin; Pfizer Animal Health, New York, NY); SP = Spectramast DC (500 mg of ceftiofur hydrochloride; Pfizer Animal Health); TM = ToMorrow Dry Cow (300 mg of cephapirin benzathine; Boehringer Ingelheim Vetmedica Inc., St. Joseph, MO).

SD): parity (2.9 ± 1.2), previous LS (3.0 ± 1.6), previous total milk (12,551 ± 3,392 kg), dry period length (54.2 ± 11.5 d), and udder hygiene score (1.7 ± 0.7) at dry-off. At the quarter level, treatment groups did not differ regarding teat end scores (1.7 ± 0.8) at dry-off. Missing information included 11 cows with missing previous LS and previous total milk (QT: n = 7, SP: n = 2, and TM: n = 2), 27 cows with missing dry period lengths (QT: n = 10, SP: n = 10, and TM: n = 7) and 15 quarters with missing teat end scores (QT: n = 9, SP: n = 0, and TM: n = 6). Previous total milk was excluded for 1 cow due to an unrealistic reported value of 117 kg.

IMI Status at Dry-Off

A total of 4,260 quarters were used for analysis of risk for presence of infection at dry-off, due to 13 missed samples (QT: n = 5, SP: n = 5, and TM: n = 3) and 91 contaminated samples (QT: n = 28, SP: n = 33: and TM: n = 30). The overall crude prevalence of IMI at dry-off was 19.2% (Table 2) and was not different among treatments [LSM: QT = 0.22 (95% CI: 0.18, 0.26), SP = 0.21 (95% CI: 0.18, 0.26), and TM = 0.23 (95% CI: 0.19, 0.27); $P = 0.73$]. Significant covariates in the model predicting presence of IMI at dry-off included region ($P < 0.01$), previous LS ($P < 0.01$), teat end score at dry-off ($P < 0.01$), udder hygiene score at dry-off ($P = 0.04$), and parity ($P < 0.01$; model not shown). The pathogen most commonly isolated from milk samples at dry off was CNS, representing 53.9% of all isolates recovered, followed by *Aerococcus* spp. (12.3%) and *Streptococcus* spp. (7.4%). Gram-positive organisms, gram-negatives and "others" represented 94.4, 4.9, and 0.7% of all organisms isolated, respectively (Table 3).

Effect of Treatment on Risk for Presence of an IMI at 0 to 6 DIM (S2)

A total of 4,058 quarters were used in the analysis of risk for presence of infection at 0 to 6 DIM (S2). From the 4,364 quarters initially enrolled, 108 quarters (27 cows) were from cows that either were culled or died during the dry period (QT: n = 40, SP: n = 40, and TM: n = 28), 99 quarters were from cows that did not have their first postcalving sample collected (QT: n = 20, SP: n = 47, and TM: n = 32), 16 quarters were from cows that died between calving and their first postcalving sample (QT: n = 4, SP: n = 4, and TM: n = 8), 8 quarters were from cows that were culled between calving and their first postcalving sample (QT: n = 4, SP: n = 4, and TM: n = 0) and 75 samples were contaminated (QT: n = 26, SP: n = 27, and TM: n = 22).

The overall crude proportion of quarters with an IMI present at S2 (0 to 6 DIM) was 14.7% (Table 2), with no difference among the 3 treatments [LSM: QT = 0.16 (95% CI: 0.14, 0.19), SP = 0.14 (95% CI: 0.12, 0.17), and TM = 0.16 (95% CI: 0.14, 0.19); $P = 0.34$]. In addition to treatment, the variables describing region ($P < 0.01$), previous LS ($P < 0.01$), and teat end score at dry-off ($P = 0.01$) were kept in the final model (Table 4).

The most common pathogen isolated at the first sampling postcalving (S2) was CNS (44.6% of all isolates recovered), followed by *Aerococcus* spp. (15.5%) and *Bacillus* spp. (10.0%). Gram-positives, gram-negatives, and "others" represented 89.7, 7.0, and 3.3% of all pathogens recovered, respectively (Table 3). Despite the fact that a priori sample size calculations were not completed to allow for subgroup analysis, the effect of treatment on prevalence of an IMI postcalving was also modeled separately for presence of IMI caused by gram-

Table 3. Description and frequency (no., with percentages in parentheses) of bacterial species for quarters that had an infection present at dry-off and at 0 to 6 DIM[1]

	IMI present at dry-off				IMI present at 0 to 6 DIM			
Item	QT	SP	TM	Total	QT	SP	TM	Total
Bacteria								
Gram-positive								
Aerococcus spp.	33 (10.9)	40 (14.3)	40 (11.9)	113 (12.3)	45 (18.1)	29 (14.4)	33 (13.9)	107 (15.5)
Bacillus spp.	21 (7.0)	25 (8.9)	17 (5.1)	63 (6.9)	31 (12.4)	23 (11.4)	15 (6.3)	69 (10.0)
CNS	175 (57.9)	135 (48.2)	184 (54.9)	494 (53.9)	108 (43.4)	94 (46.5)	105 (44.1)	307 (44.6)
Corynebacterium spp.	20 (6.6)	18 (6.4)	26 (7.8)	64 (7.0)	6 (2.4)	7 (3.5)	9 (3.8)	22 (3.2)
Enterococcus spp.	4 (1.3)	8 (2.9)	6 (1.8)	18 (2.0)	9 (3.6)	6 (3.0)	5 (2.1)	20 (2.9)
Other gram-positive	1 (0.3)	4 (1.4)	3 (0.9)	8 (0.9)	0 (0.0)	1 (0.5)	6 (2.5)	7 (1.0)
Other *Streptococcus* spp.	19 (6.3)	23 (8.2)	26 (7.8)	68 (7.4)	18 (7.2)	13 (6.4)	23 (9.7)	54 (7.8)
Staphylococcus aureus	5 (1.7)	9 (3.2)	9 (2.7)	23 (2.5)	4 (1.6)	4 (2.0)	1 (0.4)	9 (1.3)
Streptococcus dysgalactiae	1 (0.3)	6 (2.1)	4 (1.2)	11 (1.2)	2 (0.8)	6 (3.0)	6 (2.5)	14 (2.0)
Streptococcus uberis	2 (0.7)	0 (0.0)	2 (0.6)	4 (0.4)	3 (1.2)	3 (1.5)	3 (1.3)	9 (1.3)
Total gram positives	281 (93.0)	268 (95.7)	317 (94.6)	866 (94.4)	226 (90.8)	186 (92.1)	206 (86.6)	618 (89.7)
Gram-negative								
Escherichia coli	2 (0.7)	1 (0.4)	2 (0.6)	5 (0.5)	9 (3.6)	7 (3.5)	3 (1.3)	19 (2.8)
Enterobacter spp.	1 (0.3)	0 (0.0)	4 (1.2)	5 (0.5)	0 (0.0)	0 (0.0)	2 (0.8)	2 (0.3)
Klebsiella spp.	5 (1.7)	1 (0.4)	5 (1.5)	11 (1.2)	0 (0.0)	1 (0.5)	1 (0.4)	2 (0.3)
Other gram-negative	7 (2.3)	5 (1.8)	3 (0.9)	15 (1.6)	7 (2.8)	5 (2.5)	9 (3.8)	21 (3.0)
Serratia spp.	3 (1.0)	3 (1.1)	3 (0.9)	9 (1.0)	1 (0.4)	0 (0.0)	3 (1.3)	4 (0.6)
Total gram negatives	18 (6.0)	10 (3.6)	17 (5.1)	45 (4.9)	17 (6.8)	13 (6.4)	18 (7.6)	48 (7.0)
Other organisms (yeast)	3 (1.0)	2 (0.7)	1 (0.3)	6 (0.7)	6 (2.4)	3 (1.5)	14 (5.9)	23 (3.3)
Total	302	280	335	917	249	202	238	689

[1]QT = Quartermaster (1,000,000 IU of procaine penicillin G and 1 g of dihydrostreptomycin; Pfizer Animal Health, New York, NY); SP = Spectramast DC (500 mg of ceftiofur hydrochloride; Pfizer Animal Health); TM = ToMorrow Dry Cow (300 mg of cephapirin benzathine; Boehringer Ingelheim Vetmedica Inc., St. Joseph, MO).

positive or gram-negative organisms. These analyses showed no effect of treatment on risk for presence of gram-positive IMI or risk for presence of gram-negative IMI (models not shown).

Effect of Treatment on Risk for Experiencing a Cure Between Dry-Off and Postcalving

A total of 835 quarters had an IMI present at dry-off and so were at risk for a cure. However, quarters for which samples were contaminated or missing for any 1 of the 2 samples postcalving (S2 or S3) could not be assigned a cure status and, therefore, were not included in the analysis. Out of the initially eligible quarters, a total of 11 samples were contaminated (QT: n = 5, SP: n = 2, and TM: n = 4) and 41 missing samples (QT: n = 10, SP: n = 17, and TM: n = 14) for samples collected at 0 to 6 DIM, and 15 contaminated samples (QT: n = 8, SP: n = 5, and TM: n = 2) and 27 missing samples (QT: n = 11, SP: n = 12, and TM: n = 4) for samples collected at 7 to 13 DIM. Therefore, 741 quarters were included in the final analysis. Overall, the crude proportion of quarters experiencing a cure between dry-off and postcalving was 88.9% (Table 5), with no difference among the 3 treatment groups [LSM: QT = 0.93 (95% CI: 0.87, 0.97), SP = 0.93 (95% CI: 0.86, 0.96), and TM = 0.94 (95% CI: 0.89, 0.97); $P = 0.79$]. Teat end score at dry-off ($P < 0.01$) and teat end score at S3 ($P = 0.04$) were associated with risk for cure (Table 6). Although this final model defined cure as the

Table 4. Final multivariate logistic regression model for the analysis of odds for presence of an IMI at 0 to 6 DIM

Variable[1]	Coefficient	SE	Odds ratio[2]	95% CI[3]		*P*-value
				LCL	UCL	
Intercept	−1.67	0.18				
Treatment						
QT	0.04	0.12	1.04	0.81	1.32	0.34
SP	−0.15	0.13	0.86	0.67	1.12	
TM	Referent		1.00			
Region						
CA	−0.46	0.12	0.63	0.50	0.81	<0.01
IA	−0.21	0.13	0.81	0.63	1.05	
MN	Referent		1.00			
Teat score at S1[4]						
1 and 2	−0.33	0.13	0.72	0.56	0.93	0.01
3 and 4	Referent		1.00			
Previous LS[5]	0.14	0.03	1.15	1.08	1.22	<0.01

[1]QT = Quartermaster (1,000,000 IU of procaine penicillin G and 1 g of dihydrostreptomycin; Pfizer Animal Health, New York, NY); SP = Spectramast DC (500 mg of ceftiofur hydrochloride; Pfizer Animal Health); TM = ToMorrow Dry Cow (300 mg of cephapirin benzathine; Boehringer Ingelheim Vetmedica Inc., St. Joseph, MO); CA = California; IA = Iowa; MN = herds from both states of Minnesota and Wisconsin.

[2]Odds for presence of an IMI.

[3]Confidence interval for the odds ratio: lower (LCL) and upper (UCL) confidence limits.

[4]Sample 1 (S1) corresponds to the dry-off event.

[5]Last linear score (LS) before dry-off.

failure to culture bacterial pathogens in both samples postcalving, separate models that defined cure as the failure to culture a bacterial pathogen between dry-off and either the first or the second sampling postcalving found similar results (models not shown).

The effect of treatment on this primary outcome was also evaluated using noninferiority analysis by constructing a figure containing the confidence interval for the treatment effect and both the margins of inferiority and the null effect (Piaggio et al., 2006). Because the

Table 5. Crude quarter-level bacteriological cures by pathogen group

Item	Cure[1]			
	QT	SP	TM	Total
Quarters at risk for a cure (no.)	243	217	281	741
Quarters experiencing a cure [% (no.)]	88.9 (216)	88.0 (191)	89.7 (252)	88.9 (659)
Gram-positive[2] [% (no.)]				
Aerococcus spp.	93.3 (30)	88.9 (36)	97.4 (39)	93.3 (105)
Bacillus spp.	94.7 (19)	87.0 (23)	88.2 (17)	89.8 (59)
CNS	81.7 (153)	82.0 (111)	84.5 (168)	82.9 (432)
Corynebacterium spp.	100.0 (13)	100.0 (18)	100.0 (24)	100.0 (55)
Enterococcus spp.	100.0 (4)	100.0 (8)	100.0 (5)	100.0 (17)
Other gram-positive	100.0 (1)	100.0 (4)	100.0 (3)	100.0 (8)
Other *Streptococcus* spp.	100.0 (16)	84.2 (19)	77.3 (22)	86.0 (57)
Staphylococcus aureus	80.0 (5)	42.9 (7)	88.9 (9)	71.4 (21)
Streptococcus dysgalactiae	—	60.0 (5)	75.0 (4)	66.7 (9)
Streptococcus uberis	100.0 (1)	—	100.0 (2)	100.0 (3)
Gram-negative[2] [% (no.)]				
Escherichia coli	100.0 (2)	100.0 (1)	100.0 (1)	100.0 (4)
Enterobacter spp.	100.0 (1)	—	100.0 (4)	100.0 (5)
Klebsiella spp.	100.0 (5)	—	80.0 (5)	90.0 (10)
Other gram-negative	100.0 (7)	100.0 (5)	66.7 (3)	93.3 (14)
Serratia spp.	100.0 (2)	33.3 (3)	66.7 (3)	62.5 (8)
Others[2] [% (no.)]	100.0 (2)	100.0 (1)	0.0 (1)	75.0 (4)

[1]QT = Quartermaster (1,000,000 IU of procaine penicillin G and 1 g of dihydrostreptomycin; Pfizer Animal Health, New York, NY); SP = Spectramast DC (500 mg of ceftiofur hydrochloride; Pfizer Animal Health); TM = ToMorrow Dry Cow (300 mg of cephapirin benzathine; Boehringer Ingelheim Vetmedica Inc., St. Joseph, MO).

[2]The percentage corresponds to the percentage of quarters experiencing a cure; the number is the number of quarters at risk for each category.

confidence interval for TM is wholly between the margins of inferiority and includes zero, we confirm that treatment with TM is noninferior to both treatments with QT and SP (Figures 1 and 2).

Despite the fact that a priori sample size calculations were not completed to allow for subgroup analysis, the effect of treatment on cure of an IMI was also modeled separately for cure of IMI caused by gram-positive or gram-negative organisms, and these analyses showed no effect of treatment on risk for cure of a gram-positive IMI or risk for cure of a gram-negative IMI (models not shown). It is important to mention that the small sample size for analysis of cure of an IMI caused by gram-negative pathogens does not allow for conclusions.

Effect of Treatment on Risk for Developing an NIMI Between Dry-Off and 0 to 6 DIM (S2)

All quarters enrolled were considered at risk for developing an NIMI over the dry period. However, quarters that had contaminated or missing samples at dry-off (S1) or at the first sampling postcalving (S2) were not assigned a new infection status and were, therefore, excluded from analysis. A total of 3,962 quarters were used for analysis of effect of treatment on new infections. A total of 402 quarters were not eligible for the analysis. Of these, 165 quarters were excluded due to contaminated samples (QT: n = 55, SP: n = 60, and TM: n = 50) and 237 quarters were excluded due to missing samples (QT: n = 71, SP: n = 97, and TM: n = 69).

The overall crude proportion of eligible quarters developing an NIMI between dry-off and 0 to 6 DIM was 13.3% (Table 7). No effect was observed of treatment on risk for developing an NIMI [LSM: QT = 0.15 (95% CI: 0.12, 0.18), SP = 0.12 (95% CI: 0.10, 0.15), and TM = 0.14 (95% CI: 0.12, 0.17); $P = 0.27$]. Region ($P < 0.01$), previous LS ($P = 0.02$), teat end score at dry-off ($P = 0.02$), and parity ($P = 0.03$) were associated with risk for developing an NIMI between dry-off and 0 to 6 DIM (Table 8). The effect of treatment on risk for developing an NIMI between dry-off and 7 to 13 DIM was also modeled separately and showed no difference among the 3 treatments (model not shown). Despite the fact that a priori sample size calculations were not completed to allow for subgroup analysis, the effect of treatment on risk for NIMI was also modeled separately for NIMI caused by gram-positive or NIMI caused by gram-negative organisms and these analyses showed no effect of treatment on risk for a gram-positive NIMI or risk for a gram-negative NIMI (models not shown). It is important to note that the small sample size for analysis of NIMI caused by gram-negative pathogens does not allow for conclusions.

Table 6. Final multivariable logistic regression model for the analysis of odds for experiencing a cure between dry-off and calving

Variable[1]	Coefficient	SE	Odds ratio[2]	95% CI[3]		*P*-value
				LCL	UCL	
Intercept	3.62	0.66				
Treatment						
QT	−0.10	0.30	0.90	0.50	1.63	0.79
SP	−0.21	0.31	0.81	0.44	1.47	
TM	Referent		1.00			
Region						
CA	−0.41	0.28	0.67	0.38	1.16	0.30
IA	−0.41	0.35	0.66	0.33	1.31	
MN	Referent		1.00			
Teat score at S1[4]						
1 and 2	−1.87	0.61	0.15	0.05	0.51	<0.01
3 and 4	Referent		1.00			
Teat score at S3[5]						
1 and 2	0.65	0.32	1.92	1.03	3.60	0.04
3 and 4	Referent		1.00			

[1]QT = Quartermaster (1,000,000 IU of procaine penicillin G and 1 g of dihydrostreptomycin; Pfizer Animal Health, New York, NY); SP = Spectramast DC (500 mg of ceftiofur hydrochloride; Pfizer Animal Health); TM = ToMorrow Dry Cow (300 mg of cephapirin benzathine; Boehringer Ingelheim Vetmedica Inc., St. Joseph, MO); CA = California; IA = Iowa; MN = herds from both states of Minnesota and Wisconsin.

[2]Odds of experiencing a cure.

[3]Confidence interval for the odds ratio: lower (LCL) and upper (UCL) confidence limits.

[4]Sample 1 (S1) corresponds to the dry-off event.

[5]Sample 3 (S3) corresponds to the postcalving sampling collected between 7 and 13 DIM.

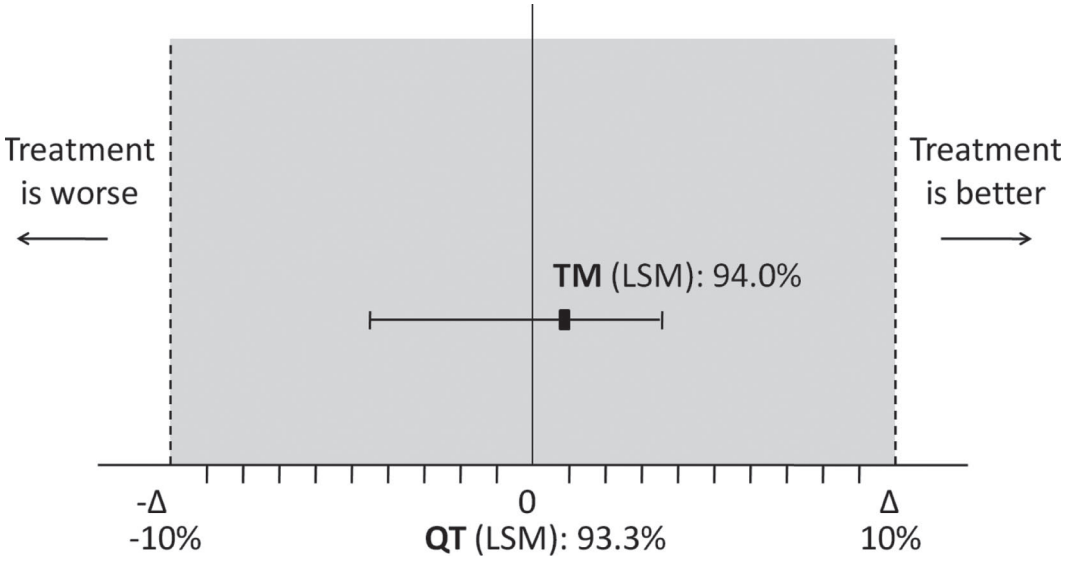

Figure 1. Noninferiority analysis of risk for cure for quarters from cows treated with ToMorrow Dry Cow (TM; Boehringer Ingelheim Vetmedica Inc., St. Joseph, MO; LSM = 0.94; 95% CI: 0.89 to 0.97) compared with cows treated with Quartermaster (QT; Pfizer Animal Health, New York, NY; LSM = 0.93; 95% CI: 0.87 to 0.97). The error bars indicate 2-sided 95% confidence intervals and the shaded area indicates the zone of noninferiority. Delta (Δ) represents the margin of noninferiority, preestablished at 10%.

Effect of Treatment on Risk for Experiencing a Clinical Mastitis Event Between Calving and 100 DIM

For the survival analysis using the Cox proportional hazards regression model, 4,232 quarters were used, with a total of 24 quarters excluded from analysis due to missing previous LS information (QT: n = 12, SP: n = 4, and TM: n = 8). Overall, 4.4% of the quarters experienced a clinical mastitis event from calving to 100 DIM. This analysis showed no effect of treatment on risk or days to a clinical mastitis event by 100 DIM (crude proportions: QT = 5.3%, SP = 3.8%, and TM = 4.1%: $P = 0.27$). Other covariates significant in the multivariate model included region ($P < 0.01$), previous LS ($P < 0.01$), and parity ($P = 0.03$: Table 9).

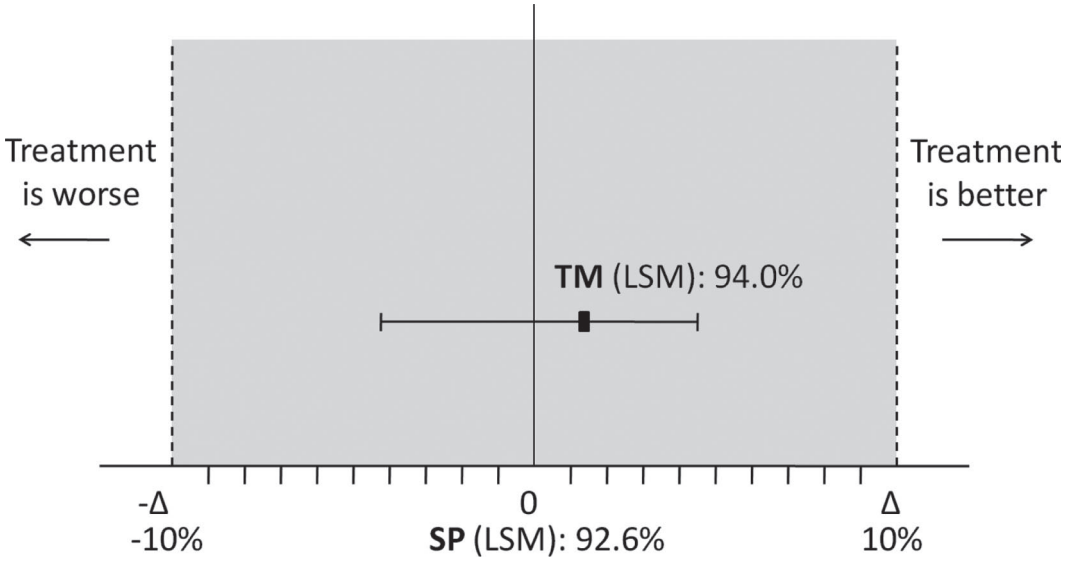

Figure 2. Noninferiority analysis of risk for cure for quarters from cows treated with ToMorrow Dry Cow (TM; Boehringer Ingelheim Vetmedica Inc., St. Joseph, MO; LSM = 0.94; 95% CI: 0.89 to 0.97) compared with cows treated with Spectramast DC (SP; Pfizer Animal Health, New York, NY; LSM = 0.93; 95% CI: 0.86 to 0.96). The error bars indicate 2-sided 95% confidence intervals and the shaded area indicates the zone of noninferiority. Delta (Δ) represents the margin of noninferiority, preestablished at 10%.

Table 7. Crude quarter-level risk for new infections between dry-off and 0 to 6 DIM

Item	New infection[1]			
	QT	SP	TM	Total
Quarters at risk for the event (no.)	1,366	1,239	1,357	3,962
Quarters with a new IMI [% (no.)]	14.1 (192)	11.9 (148)	13.8 (187)	13.3 (527)
Gram-positive[2] [% (no.)]				
Aerococcus spp.	2.8 (1,334)	2.9 (1,201)	2.4 (1,318)	2.7 (3,853)
Bacillus spp.	1.3 (1,346)	0.8 (1,215)	0.5 (1,340)	0.9 (3,901)
CNS	7.2 (1,201)	7.8 (1,115)	6.8 (1,185)	7.2 (3,501)
Corynebacterium spp.	0.7 (1,349)	0.8 (1,221)	1.1 (1,332)	0.8 (3,902)
Enterococcus spp.	0.4 (1,362)	0.2 (1,231)	0.2 (1,352)	0.3 (3,945)
Other gram-positive	0.1 (1,365)	0.2 (1,235)	0.2 (1,354)	0.2 (3,954)
Other *Streptococcus* spp.	1.7 (1,348)	1.5 (1,226)	1.2 (1,339)	1.5 (3,913)
Staphylococcus aureus	0.1 (1,361)	0.2 (1,232)	0.1 (1,348)	0.1 (3,941)
Streptococcus dysgalactiae	0.2 (1,366)	0.3 (1,234)	0.2 (1,353)	0.3 (3,953)
Streptococcus uberis	0.3 (1,365)	0.3 (1,239)	0.3 (1,355)	0.3 (3,959)
Gram-negative[2] [% (no.)]				
Escherichia coli	0.4 (1,364)	0.2 (1,238)	0.4 (1,356)	0.4 (3,958)
Enterobacter spp.	0.0 (1,365)	0.0 (1,239)	0.0 (1,353)	0.0 (3,957)
Klebsiella spp.	0.0 (1,364)	0.0 (1,236)	0.1 (1,352)	0.0 (3,952)
Other gram-negative	0.4 (1,364)	0.5 (1,236)	0.4 (1,355)	0.5 (3,955)
Serratia spp.	0.1 (1,363)	0.2 (1,236)	0.1 (1,354)	0.1 (3,953)
Other organisms[2] [% (no.)]	0.5 (1,365)	0.2 (1,237)	0.4 (1,356)	0.4 (3,958)

[1]QT = Quartermaster (1,000,000 IU of procaine penicillin G and 1 g of dihydrostreptomycin; Pfizer Animal Health, New York, NY); SP = Spectramast DC (500 mg of ceftiofur hydrochloride; Pfizer Animal Health); TM = ToMorrow Dry Cow (300 mg of cephapirin benzathine; Boehringer Ingelheim Vetmedica Inc., St. Joseph, MO).

[2]The percentage is the percentage of quarters developing a new IMI and the number is the number of quarters at risk of acquiring a new IMI for each pathogen, excluding quarters that already had the specific pathogen present at dry-off.

Table 8. Final multivariable logistic regression model for the analysis of effect of treatment on odds for acquiring a new IMI between dry-off and 0 to 6 DIM

Variable[1]	Coefficient	SE	Odds ratio[2]	95% CI[3]		*P*-value
				LCL	UCL	
Intercept	−1.46	0.21				
Treatment						
QT	0.05	0.13	1.05	0.81	1.35	0.27
SP	−0.17	0.13	0.85	0.65	1.11	
TM	Referent		1.00			
Region						
CA	−0.65	0.13	0.52	0.41	0.68	<0.01
IA	−0.25	0.13	0.78	0.60	1.01	
MN	Referent		1.00			
Parity						
2	−0.25	0.12	0.78	0.62	0.98	0.03
>2	Referent		1.00			
Teat score at S1[4]						
1 and 2	−0.31	0.14	0.73	0.56	0.96	0.02
3 and 4	Referent		1.00			
Previous LS[5]	0.08	0.04	1.09	1.01	1.17	0.02

[1]QT = Quartermaster (1,000,000 IU of procaine penicillin G and 1 g of dihydrostreptomycin; Pfizer Animal Health, New York, NY); SP = Spectramast DC (500 mg of ceftiofur hydrochloride; Pfizer Animal Health); TM = ToMorrow Dry Cow (300 mg of cephapirin benzathine; Boehringer Ingelheim Vetmedica Inc., St. Joseph, MO); CA = California; IA = Iowa; MN = herds from both states of Minnesota and Wisconsin.

[2]Odds of acquiring a new infection.

[3]Confidence interval for the odds ratio: lower (LCL) and upper (UCL) confidence limits.

[4]Sample 1 (S1) corresponds to the dry-off event.

[5]Last linear score (LS) before dry-off.

Table 9. Final Cox proportional hazards regression model for the analysis of effect of treatment on risk for experiencing a clinical mastitis event between calving and 100 DIM

Variable[1]	Coefficient	SE	Hazards ratio[2]	95% CI[3]		*P*-value
				LCL	UCL	
Treatment						
QT	0.27	0.21	1.31	0.87	1.947	0.27
SP	−0.05	0.21	0.95	0.63	1.44	
TM	Referent		1.00			
Region						
MN	1.13	0.20	3.10	2.09	4.60	<0.01
IA	−1.26	0.38	0.28	0.13	0.60	
CA	Referent		1.00			
Previous LS[4]	0.17	0.05	1.19	1.08	1.30	<0.01
Parity						
2	−0.39	0.18	0.68	0.48	0.96	0.03
>2	Referent		1.00			

[1]QT = Quartermaster (1,000,000 IU of procaine penicillin G and 1 g of dihydrostreptomycin; Pfizer Animal Health, New York, NY); SP = Spectramast DC (500 mg of ceftiofur hydrochloride; Pfizer Animal Health); TM = ToMorrow Dry Cow (300 mg of cephapirin benzathine; Boehringer Ingelheim Vetmedica Inc., St. Joseph, MO); CA = California; IA = Iowa; MN = herds from both states of Minnesota and Wisconsin.

[2]Hazard of experiencing a clinical mastitis event.

[3]Confidence interval for the odds ratio: lower (LCL) and upper (UCL) confidence limits.

[4]Last linear score (LS) before dry-off.

Out of the total numbers of quarters that experienced a clinical case from calving to 100 DIM, 51.6% of the milk samples were missing (i.e., not collected by herd staff), 23.1% yielded no growth, 2.7% corresponded to mixed infections, and 1.6% corresponded to contaminated samples. Of the milk samples for which bacteria were isolated, the majority of IMI were caused by *Escherichia coli* (22.4%), followed by *Strep. uberis* (12.2%), and other *Streptococcus* spp. (12.2%).

DISCUSSION

The current study found that TM was noninferior in effecting the primary outcome of bacteriological cure compared with QT or SP. The study also found no difference in efficacy between the 3 DCT treatments when evaluated at the quarter level for risk for presence of IMI at 0 to 6 DIM, risk for development of NIMI between dry-off and 0 to 6 DIM, and risk for a clinical mastitis event between calving and 100 DIM.

This is the first prospective multi-state, multi-herd noninferiority North American study specifically designed to compare efficacy among 3 commonly used commercial DCT products. It is difficult to compare results from this study to previous research because peer-reviewed publications directly comparing those 3 or any other DCT products are almost entirely lacking. A randomized trial conducted in 2006 evaluated the efficacy of both cephapirin benzathine and ceftiofur hydrochloride to treat existing IMI and prevent NIMI during the dry period (Hallberg et al., 2006). However, the latter study was designed to compare these antimicrobial treatments against a negative control group, and not each other, and so lacked a sufficient sample size to compare outcomes between the 2 antimicrobials tested. Furthermore, that study enrolled only cows with a SCC >400,000 cells/mL, which is not necessarily representative of the target population, considering that most dairy producers in North America apply DCT to all cows at dry-off. A noninferiority study was recently conducted in New Zealand with the aim of comparing the efficacy of 2 cephalonium products (McDougall, 2010). However, because that study had geographical differences and used different drug formulations, it does not allow for comparison with the present US study. A study conducted in Florida (Pinedo et al., 2012) compared SP and QT but did not report quarter-level outcomes, so comparisons cannot be made with the current study.

A strength of the present study is that it was conducted in commercial dairy herds from 4 different states, using different dry cow housing and management strategies. Also, the types and frequencies of pathogens recovered were very similar to those reported in other North American dry cow mastitis studies. However, it must be acknowledged that the herds used in this study were larger than average (2,230 lactating cows), compared with the average number of lactating cows in US dairy herds (167 lactating cows; USDA-NAHMS, 2010) and compared with herds that are enrolled in Min-

nesota DHIA (129 lactating cows; Minnesota DHIA, 2011). Study herds also had higher rolling herd average (**RHA**) and lower average SCC (RHA = 12,360 kg and SCC = 242,170 cells/mL) compared with Minnesota DHIA herds (RHA = 9,600 kg and SCC = 304,000 cells/mL; Minnesota DHIA, 2011). Finally, all study herds were using an internal teat sealant (Orbeseal; Pfizer Animal Health) and some kind of commercial coliform mastitis vaccine at dry-off. The 2007 NAHMS study (USDA-NAHMS, 2008) reported that approximately 30% of all operations and approximately 49% of larger operations with ≥500 cows routinely use an internal teat sealant. Similarly, approximately 38% of all operations reported vaccinating for coliform mastitis (USDA-NAHMS, 2010). As such, although results are generalizable to herds similar to those used in the current study, future studies could investigate if findings are similar in smaller herds and (or) herds not routinely using internal teat sealants at dry-off.

IMI Status at Dry-Off and Effect of Treatment on Risk for Presence of an IMI at 0 to 6 DIM (S2)

The current study found no effect of treatment on risk for presence of an IMI at dry-off or after calving. The crude prevalence of infection at dry-off reported in this study (19.2%) was within the range commonly reported in other North American dry cow studies (Godden et al., 2003; Hallberg et al., 2006; Pantoja et al., 2009), even though some differences may be expected partly due to differences in IMI definitions or sampling methodology among studies. In a study of 2 Wisconsin dairies, a prevalence of IMI at dry-off of approximately 32% was reported when an IMI was defined as the presence of 1 colony in 0.1 mL of milk for any pathogen (Godden et al., 2003), whereas 12.8% was reported in another study of 1 Wisconsin herd where the threshold of presence of 3 or more colonies in 0.01 mL of milk was required to define an IMI (Pantoja et al., 2009). Postcalving prevalence of IMI reported in recent dry cow mastitis studies is highly variable. Prevalence of infection from 2 to 9 DIM for quarters from cows treated with penicillin/dihydrostreptomycin has been reported as 6.9% (Pantoja et al., 2009), whereas a different study reported a postcalving prevalence of IMI of 40.4% for quarters treated with ceftiofur hydrochloride and 44.5% for quarters treated with cephapirin benzathine (Hallberg et al., 2006). However, the latter study enrolled only high-somatic cell cows at dry-off, and so may have included more chronic infections. As such, one might expect quarters to be more likely to have subclinical infections present after calving. Postcalving prevalence of IMI in the current study (14.7%) is relatively similar to that reported by Godden et al. (2003), wherein IMI prevalence rates of 22.8 and 20.6% were reported at 1 to 3 and 6 to 8 DIM, respectively.

Similar to previous dry cow mastitis studies, the pathogen most commonly isolated for all sampling events in the current study was CNS. Hallberg et al. (2006) reported that 62.6% of the pathogens isolated at dry-off were CNS, and Pantoja et al. (2009) reported that CNS was responsible for 63 and 44% of the infections at dry-off and postcalving, respectively. The high frequency of environmental *Streptococcus* spp. found in the current study differs from some other dry cow studies, wherein lower frequencies were reported (Pantoja et al., 2009; Gundelach et al., 2011), but it is similar to what was reported by Godden et al. (2003). Interestingly, *Bacillus* spp. were isolated in pure culture consistently in dry-off and postcalving samples from all herds in all regions. The role of *Bacillus* spp. in subclinical IMI is not well established in the literature. *Bacillus* spp. are known to be incriminated as a cause of clinical mastitis (Nieminen et al., 2007) and are also reported to be found in the normal bovine teat microflora (Al-Qumber and Tagg, 2006). We speculate that this pathogen might be considered a common contaminant by many microbiology laboratories and is, therefore, underreported in routine laboratory results. An alternative explanation may be that the prevalence of this organism may be increasing. The latter hypothesis may be supported by findings from a recent study wherein 4% of clinical mastitis cases were caused by *Bacillus* spp. (Lago et al., 2011). Further research is required to investigate the relationship between *Bacillus* spp. and udder health and disease.

In the current study, relatively few gram-negative pathogens and very few contagious pathogens (*Staph. aureus* and *Strep. agalactiae*) were reported. Two previous studies reported the proportion of IMI caused by gram-negative pathogens in dry off samples to be approximately 0.25% (Pantoja et al., 2009), 1.5% (Hallberg et al., 2006), and 22% (Godden et al., 2003), whereas the proportion of IMI postcalving caused by gram-negative pathogens were reported as 0.86% (Pantoja et al., 2009) and 30% (Godden et al., 2003). Despite these highly variable rates of subclinical IMI, studies consistently report that the prevalence of subclinical coliform IMI during lactation is highest shortly after calving and tends to decrease as DIM advances (Hogan and Smith, 1998). The low number of *Staph. aureus* and *Strep. agalactiae* common to all study herds in the present study very likely reflects good overall mastitis-control programs, including the implementation of the

Five-Point Program, a program developed at the National Institute for Research in Dairying (Reading, UK) that was adopted by progressive herds to control and prevent IMI caused by contagious pathogens. Adoption of such methods has resulted in great progress over the years, and in this scenario other pathogens such as CNS and environmental *Streptococcus* spp. have become relatively more important. Another fact that might help to explain the low prevalence of *Staph. aureus* is the poor sensitivity to detect this pathogen in a single quarter milk sample (Sears et al., 1990). The current study did not report any *Strep. agalactiae*, which is similar to findings from other North American dry cow mastitis studies (Godden et al., 2003; Hallberg et al., 2006; Pantoja et al., 2009).

Effect of Treatment on Risk for Experiencing a Cure Between Dry-Off and Postcalving

The current study found that TM was noninferior to QT or SP on risk for experiencing cure of an IMI during the dry period. The crude proportion of quarters experiencing a cure in this study (88.9%) was similar to that reported in recent dry cow studies (Godden et al., 2003; Pantoja et al., 2009; Gundelach et al., 2011). In a previous North American study evaluating ceftiofur hydrochloride and cephapirin benzathine, cure rates from dry-off to 3 and 5 DIM were lower than those reported in the present study (61.8 and 56.3 vs. 88.0 and 89.7% for ceftiofur and cephapirin, respectively). However, the latter study only enrolled cows with a high SCC, which may have represented more chronic infections and so might be less likely to cure (Hallberg et al., 2006).

One item that must be addressed is the fact that, because the crude IMI rate at dry-off was lower than anticipated (approximately 19% instead of the anticipated 30%), fewer infected quarters were enrolled at dry-off than originally anticipated. A post-hoc power calculation estimated that the study had approximately 73% power to detect a difference (delta) of 10% in cure rates between treatment groups compared. Although this loss of power needs to be acknowledged, we do not consider this to be a serious weakness of the study, given that the numeric difference observed in cure rates was very small (observed delta $<2\%$), and numerically in favor of the TM product (LSM estimates of cure rates: TM = 94.0%, QM = 93.3%, and SP = 92.6%) . Therefore, we do not believe that the loss of power in any way compromised the validity of the conclusions reached in this study. Had the observed delta in cure rates been hovering around the -10% mark, then the reduced power for this study would be a more serious limitation.

Effect of Treatment on Risk for Developing an NIMI Between Dry-Off and 0 to 6 DIM (S2)

The current study found no effect of treatment on risk for development of NIMI postcalving. The new crude IMI rate reported in the current study (13.3%) is within the range reported in other recent studies, which have reported that NIMI incidences vary between 6 to 36% (Godden et al., 2003; Cook et al., 2005; Hallberg et al., 2006; Pantoja et al., 2009; Gundelach et al., 2011). The majority of NIMI were caused by CNS and environmental streptococci in the current study. Similar findings were reported by Godden et al. (2003) and Pantoja et al. (2009). However, the 2 latter studies had gram-negative pathogens among the 3 most common organisms causing NIMI, which was not observed in the current study.

Effect of Treatment on Risk for Experiencing a Clinical Mastitis Event Between Calving and 100 DIM

The crude incidence of clinical mastitis in early lactation in the current study (4.4%) matches the incidence rates reported in previous North American studies, which ranged between 3 to 6% (Godden et al., 2003; Gundelach et al., 2011). We were not able to adequately characterize pathogens causing all of these cases because personnel from the study herds did not consistently collect and submit milk samples from clinical cases.

Secondary Findings

Previous LS. The current study detected interesting associations between other covariates and the dependent variables of interest. As an example, the previous LS from the last DHIA test day before dry-off was positively associated with risk for presence of an IMI at dry-off, at 0 to 6 DIM and with risk for a clinical mastitis event between calving and 100 DIM. This is consistent with a previous study that reported that cows with a SCC $\geq$200,000 at dry-off and postcalving were 2.7 times more likely to experience a first case of mastitis in the first 120 DIM than quarters with SCC $<$200,000 cells/mL (Pantoja et al., 2009). In the current study, a 1-unit increase in LS before drying off was associated with a 23% increased odds of developing a clinical case between calving and 100 DIM. Previous LS was also positively associated with risk for development of an NIMI between dry-off and postcalving, which has also been previously reported by several authors (Godden et al., 2003; McDougall, 2010). Somatic cell count measure is commonly used as an indicator of udder

infection status, as it reflects the number of leukocytes moving from the bloodstream to the cow's mammary gland to fight infection. Preexisting inflammation (high SCC) and potential subclinical infection might indirectly characterize a quarter at greater risk for development of NIMI or clinical flare-ups, especially during periods when the cow is immunosuppressed, such as during the transition time.

Teat End Score. Previous reports on the nature of the association between teat end score and risk for presence or incidence of IMI are somewhat contradictory, and the relationship between teat end score and cure risk has never been previously described in the literature. Some studies found that risk for IMI in quarters with normal teat ends was not different from quarters with chronic ring lesions on teat ends (Sieber and Farnsworth, 1981). However, findings from the current study are consistent with other studies reporting that worse teat end condition is positively associated with risk for presence of subclinical infections (de Pinho Manzi et al., 2012) and that rough or cracked teat ends are a risk factor for the development of NIMI during the dry period (Dingwell et al., 2004). Counterintuitively, quarters with a teat end scored 1 or 2 at dry-off were 85% less likely to experience a cure compared with teat ends scored 3 or 4. We have no immediate explanation for this observed relationship. Quarters with teat ends that have lesions or that are keratinized could potentially be at a higher risk for development of NIMI due to the fact that pathogens may colonize these cracks and crevices, putting them in close proximity to the streak canal, and so predisposing the quarter to infection by ascending bacteria. It has been recently reported that teats with a calloused end and hyperkeratosis are characterized by a higher environmental microbial load (Paduch et al., 2012).

Udder Hygiene Score. Udder hygiene score at dry-off was positively associated with presence of an IMI at dry-off. This observation is in agreement with findings from a previous study wherein a positive association was reported between subclinical mastitis and measurements of animal hygiene (Schreiner and Ruegg, 2003). It has been proposed that udder and leg hygiene scores of cows provide evidence on the degree that teat ends are exposed to environmental mastitis pathogens, which is correlated to risk for presence of subclinical IMI (Schreiner and Ruegg, 2003).

Parity. The current study found that increasing parity was positively associated with risk for development of NIMI during the dry period and also with risk for a clinical mastitis event between calving and 100 DIM. Increasing parity has been previously reported as a risk factor for presence of IMI (Green et al., 2005), new infections (Dingwell et al., 2004; Cook et al., 2005; McDougall, 2010), and clinical cases until 120 DIM (Pantoja et al., 2009). Anatomical and intramammary defense mechanisms of cows may deteriorate with age, an example being the reduced function and increased diameter of the streak canal (Dingwell et al., 2004). Older cows are also more likely to have been previously exposed or infected with mastitis pathogens, which has been discussed as potential risk factor that can contribute to susceptibility for new infections (Pantoja et al., 2009).

Summary

The current study found that TM was noninferior in effecting a bacteriological cure compared with SP or QT, and that no difference in efficacy existed between the 3 commercial DCT products tested when considering all other quarter-level outcomes examined. These results are consistent with the fact that all 3 DCT products evaluated are labeled to be effective against one or more gram-positive organisms, and the majority of IMI detected in the current study were caused by gram-positive organisms. None of the 3 DCT products evaluated are labeled against gram-negative organisms, even though they are all recognized to have varying degrees of gram-negative activity in in vitro tests (Salmon et al., 1996; Constable and Morin, 2002; Oliver and Murinda, 2012). However, gram-negative IMI made up relatively few IMI cases in the current study.

CONCLUSIONS

Results from this noninferiority study demonstrate that, in herds using blanket Orbeseal infusion at dry-off, no difference in efficacy existed between the products QT, SP, and TM regarding risk for presence of IMI at 0 to 6 DIM, risk for experiencing a cure during the dry period, risk for developing an NIMI between dry-off and 0 to 6 DIM, and risk for experiencing a clinical mastitis event between calving and 100 DIM. Specifically, TM was noninferior in effecting a bacteriological cure compared with QT or SP. As such, dairy producers could potentially put aside concerns about differences in product efficacy, and instead base their selection decision among these 3 products on other characteristics such as milk and meat withholding time, targeted dry period length, and cost. From the point of view of promoting the prudent use of antimicrobials, as QT (penicillin/dihydrostreptomycin) and TM (cephapirin benzathine, a first-generation cephalosporin) products had similar efficacy compared with SP (ceftiofur hydrochloride, a third-generation cephalosporin),

the veterinary community might consider recommending the use of the older simpler antimicrobials as a first choice when recommending a DCT product.

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